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Original Article

ASSESSEMENT OF ANTI-INFLAMMATORY AND ANTI-ARTHRITIS ACTIVITY OF *JATROPHA GOSSYPIFOLIA* IN RATS

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ABSTRACT

Objectives: To investigate anti-inflammatory and anti-arthritic activity of latex *Jatropha gossypifolia* in rodents.

Methods: The anti-inflammatory and anti-arthrit activity of latex from *Jatropha gossypifolia* (JGL) were tested in two different vivo models namely carrageenan induced paw edema in rats and Freund adjuvant arthritis. These models represent acute inflammatory condition and chronic inflammatory condition respectively.

Results: The results of the present study showed that latex of *JGL* possessed remarkable anti-inflammatory activity in carrageenan induced edema (acute model) and antiarthritic activity in CFA induced (subchronic model) arthritis in rats.

Conclusion: The latex of the plant *Jatropha gossypifolia* possesses anti-inflammatory and anti-arthritic activity might be due to its rich flavonoids content.

Keyword: Anti-inflammatory, Anti-arthritis, *Jatropha gossypifolia.*

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. It is a protective attempt to remove the injurious stimuli as well as initiate healing process for the tissue. Inflammatory response is a series of well coordinated dynamic mechanism consisting of specific vascular humoral and cellular events that is characterized by the movement of fluids, plasma and inflammatory leukocytes (neutrophages, eosinophils, basophiles and macrophages) to the site of inflammation [1;2].

Pain, inflammation and fever in the body is due to the production of large amount of prostaglandin E2 (PGE2) by cell involved inflammation. Inflammation is regarded as protective and reparative response, however, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases [3].

Cyclooxygenase [prostaglandin-endoperoxide synthase, EC 1-14-99-1] is an enzyme involved in the metabolism of arachidonic acid and synthesis of prostanoids including potent proinflammatory prostaglandins (PGE_2, PGF_2) [4; 5]. In mammalian cells, COX exist in at least two isoforms COX-1 and COX-2. COX-1 and COX-2 involved in the process of inflammation whereas prostaglandins have the protective effect such as production of gastic mucous and mantenance of renal blood flow.

Number of anti-inflammatory agents such as 5-LOX (5-lipoxygenase) inhibitors and Non steroidal anti-inflammatory drugs (NSAIDs) has been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs shows a major problem during their clinical use [6].

Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary. The rheumatoid arthritis (RA) is a common human autoimmune disease characterized by chronic inflammation of the synovial membranes with concomitant destruction of cartilage and bone. It affects approximately 5 million people worldwide of which 50% are unable to work beyond 10 years of diagnosis. Anti-inflammatory agents are administrated as long term treatments for patients with RA. It has been reported that a number of flavonoids possess anti-inflammatory [7] and analgesic [8] activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects [9]. The literature survey revealed that several parts of plant *Jatropha gossypifolia latex* possess anti-inflammatory effect may be due to the prescence of flavonoids [10-12]. Therefore the present study is undertaken to investigate anti-inflammatory and anti-arthritic activity of latex *Jatropha gossypifolia* in rodents.

MATERIALS AND METHODS

Test compounds

1. Latex of *Jatropha gossypifolia,* the plant was collected from the Botanical Garden of S. K. B. College of Pharmacy, Kamptee, MS, India.

2. Standard drug: Diclofenac sodium administered intraperitoneally.

3. Phlogistic agent:

- Carrageenan: (1% w/v)
- Freund's complete adjuvant: (0.1 ml)

Animals

Sprague Dawley rats either sex weighing between 200-250g obtained from NCLAS, NIN, Hyderabad, India were used for the present study. Animals were housed under controlled environmental condition at (24±1°C) and humidity-controlled-(65±5%) with free access to food and water were used. All experimental procedures were carried out under strict compliance with Institutional animal ethical committee (IAEC) according to guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Treatments

Rats were allowed for 3 to 5 day acclimation period before being treated. They were selected randomly and divided into three groups with five animals in each group. The *Jatropha gossypifolia* latex was dissolved in distill water and sonicate for 15 minutes. Vehicle-treated control receives vehicle only (10 ml/kg) and 25mg/kg BW and 50mg/kg BW. The latex was given orally. After 24 hr, the number of dead animals was verified. All animals were individually observed after the latex or vehicle administered, at 5, 10, 15 and 30 min; 1, 2, 4 hrs and for 14 days.

Acute toxicity test

To test acute toxicity of *Jatropha gossypifolia* latex, the animals were given orally different doses of *J. gossypifolia* latex. In the present study, we are considering dose of 500mg/kg and 5000mg/kg of *Jatropha gossypifolia* latex.

Evaluation of anti-inflammatory and anti-arthritic activity

Following experimental models were used for evaluation of anti-inflammatory and anti-arthritic activity.

a) Acute model: Carrageenan induced paw edema in rats [13].

b) Sub-chronic model: Freund's complete adjuvant induced arthritis in rats [14].

Paw volume was measured by using Plethysmometer.

A) Carrageenan induced rat paw edema

Procedure

Rats were fasted overnight and divided in different groups of three animals in each. They were treated orally with the test compound, standard anti-inflammatory drug, Diclofenac (4mg/kg) for 30 mins before the subplanter injection of 0.1 ml of 1% carrageenan. Paw volumes were measured using plethysmometer (medi CAID, VJ Instruments) immediately (measured within 30 sec. And referred as initial paw volume) and again at 1st, and 3rd hour after challenge. The difference between these two observations gave the amount of edema developed. The percent inhibition of edema for the treated groups was calculated by following formula and compared with the control groups;

% Edema Inhibition = [1- (Vt / Vc)] X 100

Where, Vt and Vc are the mean changes of paw volume in the treated and control group respectively.

The results were expressed as mean changes of paw volume (ml) ± SEM and as percent inhibition of edema.

B) Freund's adjuvant induced arthritis in rat

Freund's adjuvant induced arthritis have been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance for the study of pathophysiology and pharmacology control of inflammatory process, as well as the evaluation of analgesic or anti-inflammatory effect of drugs [15;16]. The arthritis is induced by a sub-plantar injection of Freund's adjuvant. The denatured Mycobacterim butyricum suspended in mineral oil can be injected in the rat paw sub-plantar surface, or by intra joint. The paw volume, up to the ankle joint, was recorded before (day 0), and at 7, 14 days after the administration of adjuvant and the drugs were administered from day 1 to day 14 (at 10:00 a. m. Every day) post adjuvant administration. Measurement of the hind paws was taken for calculating the average change in volume. Average paw volume of each animal on day 0 was subtracted from the corresponding average hind paw volume on day 7 and 14, so as to obtain the absolute increase in paw volume which was expressed as mean ±SEM for three animals in a group. 0.1 ml of Freund's adjuvant (complete fraction of mycobacterium butyricum suspended in mineral oil; sigma chemical) was injected in the sub-plantar tissue of the right posterior paw. Every day animals were carefully and thoroughly inspected, by examining the affected paw and the animal's general status. The results were expressed as mean changes of paw volume (ml) ± SEM and as percent inhibition of edema.

RESULTS

Acute toxicity test

Acute toxicity study showed that the latex of *Jatropha gossypifolia* possessed safety profile as no death was observed at oral doses of 500mg/kg and 5000mg/kg in rats.

Phytochemical screening

The phytochemical screening of *Jatropha gossypifolia* latex indicated the presence of alkaloids, flavonoids, saponins, tannins, steroids and glycosides (Table-1).

Table 1: Phytochemical screening of *J. gossypifolia* latex

Name of constituents	Occurrence
Alkaloids	+ ve
Flavonoids	+ ve
Saponins	+ ve
Tannins	+ ve
Steroids	+ ve
Glycosides	+ ve
Protein	- ve

+ve =present and -ve =absent

Effect of *Jatropha gossypifolia* on carageenan induced paw edema

Jatropha gossypifolia latex (JGL) showed strong anti-inflammatory activity as evident from the significant reduction in carrageenan induced paw edema as compared to saline treated group (Table-2). The anti-inflammatory effects were prominent both at the first and at the third hour. The JGL significantly reduced the volume of edema. Percent inhibition revealed the highest activity of JGL 50 mg/kg (88.61%).

Table 2: Anti-inflammatory activity of latex of *J gossypifolia* (JGL) for paw edema and % inhibition induced by carrageenan in rats

Treatment (orally)	Dose(mg/kg)	Volume of edema in ml ±SEM at first hr	(Percentage inhibition) at first hr	Volume of edema in ml ±SEM at third hr	(Percentage inhibition) at third hr
Control	1 ml/kg	0.4553±0.005	-	1.421±0.006	-
Diclofenac sodium	4mg/kg	0.1758±0.009*	34.08%	0.1344±0.006*	75.11%
JGL	25mg/kg	0.114±0.005*	74.96%	0.3167±0.002*	77.71%
JGL	50mg/kg	0.067±0.008*	85.28%	0.1619±0.004*	88.61%

Values indicate mean paw volume and mean % inhibition SEM (n=3), * P< 0.001 vs saline

Table 3: Effect of JGL on CFA induced paw edema and % inhibition in rats.

Treatment (orally)	Dose (mg/Kg)	Vol. of edema in ml± SEM	% inhibition (day 7)	Vol. of edema in ml± SEM	% inhibition (day 14)
Control	1 ml/Kg	2.5208±0.05	---	1.3743±0.05	---
JGL	50mg/Kg	1.115±0.05*	55.77%	0.281±0.002*	79.55%

Group of rats (n=3) was fed JGL, mean % inhibition ± SEM, P< 0.001 vs saline.

Effect of *Jatropha gossypifolia* on Complete Freund Adjuvant (CFA) induced paw edema.

Jatropha gossypifolia latex (JGL) showed significant reduction in CFA induced paw edema compared to saline treated group (Table -3). It may exhibit potent anti-arthritic activity and significantly reduced the volume of edema. Percent inhibition revealed the highest activity of JGL 50 mg/kg (79.55%) on day 14.

DISCUSSION

Inflammation is a component of range of acute and chronic human diseases. Several inflammatory mediators like histamine, bradykinin, serotonin, arachidonic acid derivatives eicosanoids, cytokines etc. Are liberated in inflammation. In the present study, the anti-inflammatory activity of latex from *Jatropha gossypifolia* (JGL) was tested in two different vivo models namely carrageenan induced paw edema in rats and Freund adjuvant arthritis. These models represent acute inflammatory condition and chronic inflammatory condition respectively. It has been reported that a number of flavonoids possess anti-inflammatory [7] and analgesic [8] activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects [9]. The literature survey revealed that several parts of these plants of *Jatropha gossypifolia* latex possess anti-inflammatory effect it may be due to the presence of flavonoids. Carrageenan induced paw edema involves a biphasic event. The initial phase is attributed to the release of histamine and serotonin followed by increased vascular permeability by kinase. Second accelerating phase of swelling is due to the release of prostaglandin which is closely associated with migration of leucocytes into the inflamed site. The *JGL* at doses tested here (25mg/kg and 50 mg/kg, orally) significantly inhibited the carrageenan induced paw edema in rats. Such activities have been well documented for extraction of various related plants for example *Plumeria accutifolia, Plumeria accuminata, Jatropha curca* and *arial part of Jatropha gossypifolia.* Also there is a close resemblance between Freund's adjuvant induced polyarthritis and human arthritis. The condition is considered due to i) Delayed hypersensitivity response to mycobacterial antigen. ii) An autoimmune disease in which the responsible antigen is altered collagen and iii) A local response of tissues to the disseminated and indigestible adjuvant etc. The protective effect of latex from *JGL* was likely to be attributed to the inhibition of either one or combination of an above mechanism.

Besides the above mediatory, the role of oxygen derived free radicals in inflammatory process is well documented [17]. Free radicals are implicated in the activation of nuclear factor kappa B (NFKB) and protein kinase which induced the transcription of proinflammatory enzymes such as COX-2, nitric oxide synthase (NOS), inflammatory cytokines and tumor necrosis factor (TNF). Activation of leucocytes (e. g neutrophil and monocytes/ macrophages) during inflammation can result in the release of large amount of reactive oxygen intermediates including superoxide anion, hydrogen peroxide and hydroxyl radicals as host defense mechanisms [18]. Inflammation induced by carrageenan is accompanied by the significant increase in the output of lipid peroxides by liver. Similarly in arthritic condition, the granulocytes and macrophages accumulates in the affected area and produce large amount of superoxide and hydrogen peroxide radicals [19]. The scavengers of reactive oxygen species are known to reduce the tissue injury associated with inflammatory diseases [20] and many anti-inflammatory agents or flavonoids of plant sources act by scavenging of reactive oxygen radicals and inhibit of cellular oxidation. Although we have not tested our test substance for this property, the possibility cannot be ruled out that the free radical scavengic activity could also be the mechanism of action besides inhibition of release of inflammatory mediators, our test substance showed positive test for flavonoids as well as steroids.

CONCLUSION

Thus from the foregoing it was concluded that the latex of the plant *Jatropha gossypifolia* possesses anti-inflammatory and anti-arthritic activity might be due to its rich flavonoids content.

CONFLICT OF INTERESTS

Declared None

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